Article Addendum

Overexpression of caveolins in *Caenorhabditis elegans* induces changes in egg-laying and fecundity

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Caveolae are small plasma membrane-associated invaginations that are enriched in proteins of the caveolin family in addition to, sphingolipids, glycosphingolipids and cholesterol. Caveolae have been implicated in several endocytic and trafficking mechanisms. Mutations in caveolins have been shown to cause disease and caveolae offer one site for pathogen entry. The *Caenorhabditis elegans* genome encodes two caveolins (*cav-1* and *cav-2*); we have shown that these two proteins have distinct expression patterns. CAV-1 is found in the majority of cells in embryos and in the body-wall muscles, neurons and germ line of adult worms. CAV-2 is expressed in the intestine and is required for apical lipid trafficking. In the course of our studies, we generated several constructs to overexpress caveolins in *C. elegans*. Here we show that overexpression of *cav-1* protects against the decrease in brood size associated with the effects of heat shock and the presence of extrachromosomal arrays in heat-shocked animals. Furthermore, we show that overexpression of *cav-2* in the nervous system increases the rate of egg-laying and total number of eggs laid.

Cells utilize a range of different mechanisms to mediate trafficking between the plasma membrane and intracellular membranes. One such mechanism involves caveolae, which are small (50–100 nm) invaginations in the plasma membrane. These invaginations are unusually rich in caveolin, sphingolipids, glycosphingolipids, cholesterol and many signaling proteins.[1,2] Caveolae require caveolin for their formation and most caveolin appears to traffic to the plasma membrane; however, we and others have identified intracellular caveolin-containing bodies.[2-4] Caveolae have been implicated in the endocytosis of a range of molecules and pathogens[2,5,6] and have also been implicated in a number of signaling processes.[2] Mammals encode three caveolin proteins; caveolin-1 is widely expressed in many tissue types, the closely related caveolin-3 protein is restricted to myocytes, and caveolin-2 has been shown to require caveolin-1 for transport to the plasma membrane.[2] Caveolins have been implicated in a wide range of processes, such as: cancer, lung disease, lipid homeostasis and disease, liver regeneration and dystrophies.[2,7-16]

We have used *Caenorhabditis elegans* as a model system to explore the roles of caveolins. *C. elegans* encodes two caveolin proteins.[17] The *C. elegans* caveolin-2 protein (CAV-2) is localized to the apical membrane of intestinal cells. Ablation of *cav-2* induces abnormal trafficking of yolk proteins and uptake of lipid markers; furthermore, *cav-2* mutants suppress an intestinal phenotype induced by defective basolateral recycling in *rme-1* and *rab-10* mutants.[3] In contrast, caveolin-1 (*cav-1*) is widely expressed in eggs, and in many tissues at early larval stages but with maturation, CAV-1 becomes restricted to the germ line, nervous system, body-wall muscles, and most likely, the post-synaptic side of the neuromuscular junction.[18,19] Ablation of CAV-1 function, using dominant-negative constructs based on dystrophic mutations identified in humans, induces neurotransmission and locomotion defects that may offer insights into the pathology of certain muscular dystrophies.[18]

Overexpression of Caveolin-1 Protects Against Reduced Fecundity Caused by Extrachromosomal Arrays and Heat Shock

We found that overexpression of caveolin proteins was able to protect against deleterious effects of transgenes and heat shock in worms. In experiments designed to identify the effects of caveolin overexpression we used a heat-shock inducible promoter, *hsp16-2* (pHS),[20] which drives gene expression in a wide variety of tissues, to express the caveolin genes. pHS::*cav* constructs were introduced into *C. elegans* using standard microinjection techniques, which result in transgenes which are present on extrachromosomal arrays

(ECAs).[21] To test the efficiency of the pHS, we made pHS::*cav-1::gfp* and pHS::*cav-2::gfp* constructs. Worms incubated at 20°C had little or no detectible GFP, as measured by confocal microscopy; however, following a heat-shock of 33°C for 1 hour, we could detect GFP in most, if not all, tissues. We then used pHS::*cav-1* and pHS::*cav-2* constructs and a range of controls in heat shock experiments. In order to induce a high level of expression from the pHS::*cav-1* and pHS::*cav-2* animals, we heat-shocked animals twice daily for 1 hour at 33°C. The results in Figure 1A show that the various transgenes had little effect on brood size in untreated animals. However in heat shocked animals (Fig. 1B) we identified three effects. Firstly, the brood size of all animals is reduced compared to the untreated animals, which probably results from the temperature regimen that is likely to have many physiological effects. Secondly, in general, carrying an extrachromosomal array, for example the pHS::*GFP*, pHS::*lacZ*, pHS::*cav-2* or *gtl-1* (an array carrying a TRPM type channel gene[22] but no heat shock promoter) arrays, further reduces brood size. This suggests that the presence of transgenic arrays has a generic negative affect on fecundity. Thirdly, animals containing arrays that include pHS::*cav-1* exhibit brood sizes, which are higher than that of both the other array containing lines and the wild type animals in this environment (Fig. 1B). This suggests that the presence of *cav-1* is protective against the deleterious effect of ECA in heat-shocked animals. To confirm that *pHS::cav-1* was able to overcome the effects of any transgenic array we made animals carrying an array of both pHS::*cav-1* and pHS::*lacZ* as the latter has a low brood size when expressed alone. Again the fecundity is restored. We used an un-paired t-test to compare the total brood-size of worms carrying both the pHS::*cav-1* and pHS::*lacZ* constructs with worms carrying only the pHS::*lacZ*. Comparing strains in a non heat-shocked environment gave a p = 0.223 value; however, comparing worms from a heat-shocked environment gave a p = 0.003 value. Thus the presence of *cav-1* appears to protect animals against the deleterious effects of transgenes in heat shocked animals. Further, the presence of pHS::*cav-1* may even be able to partially compensate for the reduced brood size caused by heat shock. The mechanism of this effect is unclear. It has been shown that CAV-1 is highly dynamic in the germ line of *C. elegans*,[4] a possible site of action. However ECAs are generally silenced in the germ line. It is unclear whether the effect results from increased levels of CAV-1 in tissues which normally express the CAV-1 protein or , is due to ectopic expression. It has recently been suggested that *C. elegans* CAV-1 is unlikely to form caveolae,[23] thus making its role in this and other processes in *C. elegans* enigmatic.

Overexpression of Caveolin-2 Causes Increased Egg Laying

In a related set of experiments we overexpressed *cav-1* and *cav-2* in the nervous system of worms. We reasoned that overexpression
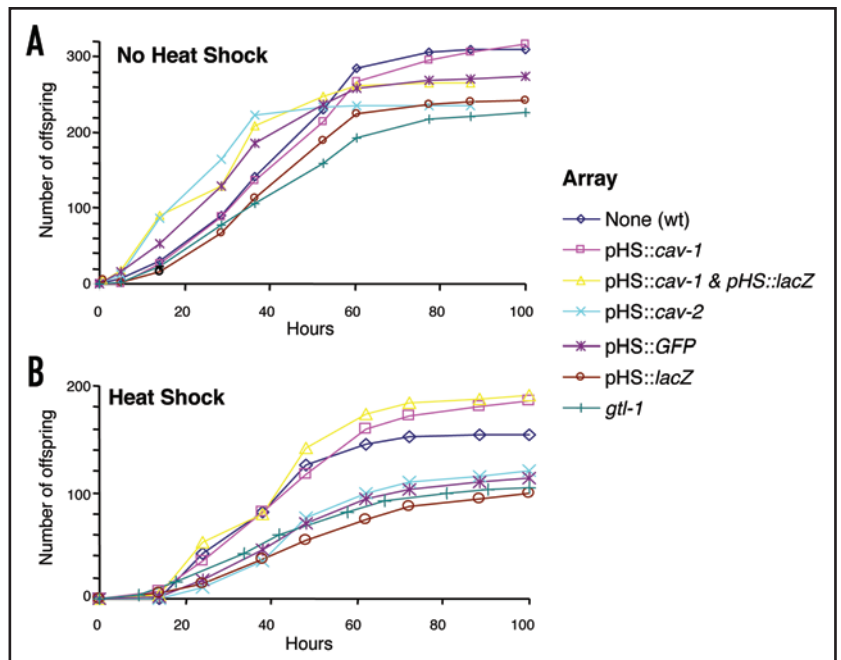

Figure 1. Overexpression of CAV-1 improves fecundity in heat-shocked worms carrying extrachromosomal arrays. Individual L4 stage worms were placed onto plates and allowed to lay eggs. N = 10 for each condition. Worms were transferred every 12 hours and the number of offspring on each plate was determined. (A) Shows the brood size of animals that received no heat-shock treatment, assayed at 20°C and (B) shows brood sizes for worms that were heat-shocked for 1 hour, twice daily, at 33°C. N2 indicates wild-type and pHS indicates the promoter from the heat-shock gene, hsp-16-2.

of *cav-1* in the nervous system, one of the main tissues expressing *cav-1*, might induce phenotypes indicative of its neuronal function and related to those we observed in animals carrying *cav-1* dominant-negative constructs.[18] To this end, we cloned the *cav-1* gene downstream from the neuron-specific promoter from the *unc-119* gene.[24] To ensure that *cav-1* was being expressed in neurons, we also made a *cav-1::gfp* construct. As a control, we made the same constructs previously described, but used the *cav-2* gene. Surprisingly, we found no obvious phenotypes in animals overexpressing *cav-1*; however, we found that *cav-2* overexpression induced an increase in the total number of eggs laid, and an increase in the rate of egg laying (data not shown). This phenotype was most obvious at 50 hours after the initiation of egg laying (*cav-1* overexpression, mean = 173, sd = 18; *cav-2* overexpression, mean = 307, sd = 37; N2 wild-type, mean = 174, sd = 14). Thus overexpression of *cav-2* from the *unc-119* promoter appears to improve fecundity in worms.

Although *cav-1* is not solely expressed in neurons, it was surprising that its overexpression there produced no detectable, neuronally-controlled, phenotypes. In contrast, overexpression of *cav-2*, a gene expressed in the intestine, did generate a phenotype when overexpressed in the nervous system. Egg laying in *C. elegans* is under neuronal control thus changes in egg laying may be the result of changes in neuronal function brought about by the presence of ectopic CAV-2 protein. The ability of CAV-2 to form caveolae has not been tested;[23] however, bioinformatic analysis

suggests that it is more likely to form caveolae than CAV-1.[3] Thus, CAV-2 may alter neuronal function by changing the trafficking in neurons or by changing the behavior of the membrane. The ability of *cav-2* to alter neuronal function in contrast to *cav-1* and the differential effects of *cav-1* and *cav-2* following heat shock further support the notion that the two proteins play different roles in cell function in *C. elegans.*

Acknowledgements

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